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synthesized and cloned in the composition of plasmid pcDNA3.1 that is eukaryotic expression vector. Accuracy of gene insertion encoding polyepitopeimmunogen was verified using restriction analysis. Obtained target plasmid pBC-A*0201 – candidate DNA-vaccine was used for evaluation of gene expression in transfected eukaryotic cells 293T and dendritic cells by means of flow cytofluorometry and immunohistochemistry using MAT to p24 epitope-marker inserted into the structure of target plasmid. Dendritic cells were obtained from peripheral blood precursors by 2-h plastic adhesion. The nonadherent cells were removed 2 h later and the adherent monolayer was cultivated in the medium containing growth factors (GM-CSF and IL-4) according to (Naik, 2010; Ganul and Khranovskaya, 2012). Transfection of immature DCs (iDC) was performed by using the reagents purchased from Promokine. Further cultivation of iDCs was carried out in the medium containing TNF- α to obtain fully mature DCs (mDC). Analysis of mDCsurface markers expression was made using MAO (HLA-DR, CD83, CD86) and flow cytofluorometer (BD FACSCalibur).

Results: Plasmid pBC-A*0201 encoding epitopes of breast cancer antigens was constructed and produced in preparative amount. Using the method of intracellular staining of product of expression by specific MAO 29F2 to epitope p24, we showed expression of gene BC-A*0201 in eukaryotic cells 293T and dendritic cells transfected by plasmid pBC-A*0201. Maturity of dendritic cells was also proved.

Conclusion: Thus, obtained candidate DNA-vaccine encoding target polyepitopeimmunogen provides synthesis of relevant protein in the eukaryotic cells culture. In future, we plan to study biological activity of DNA-vaccine to evaluate level of induction of cytotoxic response against SKBR3 breast cancer cells.

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P67

Use of extracellular RNA from urine for the diagnosis of prostate cancer

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Differently sized microparticles, including exosomes (30–100 nm), prostasomes (50–500 nm), oncosomes (50–500 nm) and other microparticles (100–1000 nm) were found in blood and urine. Exosomes from prostate cancer (PCa) patients can potentially contain cancer-specific nucleic acids, and thus can represent a valuable source of diagnostic material. In this study, we have investigated microvesicles and miRNA from urine of healthy donors and PCa patients. To isolate miRNAs from urine and microparticles, novel methods for miRNA isolation were elaborated (Rus. patent application No 2014137763, priority date 17.09.2014). The study population included 14 patients with PCa (63–82 years, T2-3NxMx1) and control group of 20 healthy volunteers with no previous history of prostate disease (48–73 years). Urine was clarified by two serial centrifugations at 400*g*, 20 °C, 20 min and at 17000*g*, 20 °C, 20 min. Microparticles were precipitated from the resulting supernatant by high-speed centrifugation at 100000*g*, 18 °C, 90 min, the pellet was resuspended and pelleted by centrifugation under the same conditions. To isolate exosomes, total microparticles were filtered through 0.1 μm pore filters and reprecipitated. The resulting pellets were resuspended and exosome samples were investigated by transmission electron microscopy (TEM). MiRNAs were isolated by one-step single-phase protocol and purified using "BioSilica" spin- columns (Zaporozhchenko et al., Anal. Biochem, upcoming, doi: 10.1016/j.ab.2015.03.028) and by recently developed method based on precipitation of excess biopolymers, allowing to isolate miRNAs with better efficiency than commercially available kits. The size and quantity assessment of extracellular RNA were performed using capillary electrophoresis system on Agilent 2100 Bioanalyzer. Concentrations of miRNAs (miR19b, miR25, miR205, miR125b, miR126) were measured by qRT-PCR and normalized to miR-16 using dCq method.

TEM demonstrated the presence of 20–300 nm microparticles in urine of healthy donors and PCa patients. Approximately 50–70% of all urine microparticles are represented by 30–100 nm exosomes and residual 30–50% by particles larger than 100 nm. (The pool of urine microvesicles consists of 50–70% exosomes and 30–50% particles larger than 100 nm.).

The major part of extracellular RNA found both in exosomes and total microparticles fraction of healthy donors and patients with PCa, is 25–200 nt long and can include tRNA (73–93 n.), 5.8 rRNA (~150 n.), snoRNK (10–20 n.), snRNA (60–300 n.) piRNAs (29–30 n.), miRNAs (20–25 n.), siRNA (21–25 n.). Concentration of extracellular urine RNA in exosomes and total microparticles of healthy donors and patients with PCa amounts to100 pg/ml of urine on average.Receiver Operating Characteristic (ROC) curve analysis of all miRNAs in samples isolated from total urine did not demonstrate any diagnostic significance. In contrast, in training cohort concentration of miR-19b in exosomes and total microparticles fraction provide 95%/75% and 93%/100% sensitivity and specificity, respectively.

Thus, microparticles, isolated from urine of PCa patients represent a valuable source of diagnostically significant miRNAs. To investigate miR-19b, a large testing cohort is required. Search for other potential miRNA-markers by microarray profiling of cell-free miRNA isolated from urine of PCa patients is also required to reveal a sold set of markers for confident PCa diagnostics.

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A111

Expressing FOXP3 in determining the biological characteristics of endometrial adenocarcinomas

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Background: According to the "immune-editing" theory, immunocompetent cells being important component of the tumor microenvironment can both show antitumor activity and contribute to the tumor progression. Tumor pathophysiological features affect the structural and functional changes of certain components of tumor microenvironment, in particular, increase the number of T-lymphocytes with regulatory activity (Treg). FOXP3, transcription factor, acts as master regulator for suppressive function of Treg. Recent studies have shown FOXP3 is expressed both in immunocompetent cells and tumor cells; however, the function of this gene in malignant tumors of different genesis is ambiguous (GirdhariLal, et al., 2014). The aim of the study was quantitative assessment of subpopulations CD4+, CD8+, FOXP3+-lymphocytes associated with the tumor, FOXP3+-tumor cells and comparison of these parameters with the clinical and morphological characteristics of endometrial cancer (EC).

Materials and methods: A total of 40 EC patients who did not receive special treatment before surgery with the mean age 56.9 ± 2.8 years were included in the study. Morphological and immunohistochemical methods were used in the study (primary monoclonal antibodies: CD4 – clone 4B12, "Millipore", USA, CD8 – clone RIV – 11, "Millipore", USA, FOXP3 – clone 5H5L12, "Invitrogen", USA, Ki-67 - clone MIB1, "DakoCytomation", Denmark) and Real-Time PCR was also used to determine the DNA methylation status of FOXP3 gene mathematical statistics.

Results: The dependence of the number of intratumoral CD4+-, CD8+- lymphocytes and FOXP3-lymphocytes on such biological characteristics as the degree of differentiation, growth rate and depth of invasion into the myometrium was established. In endometrial adenocarcinomas, low grade content of FOXP3+ lymphocytes increased (27.8 ± 2.6%), number of intratumoral CD4+ (15,3 ± 0,2%), CD8+-lymphocytes (29.6 ± 0.3%) and FOXP3+-tumor cells (15,5 ± 3,3%) decreased in contrast to the same parameters in high grade tumors: FOXP3+-lymphocytes (17.4 ± 3.0%), CD4+ (52.0 ± 2.7%), CD8+ (46.4 ± 5.6%), FOXP3+-tumor cells (27.8 ± 2.6%), $p < 0.05$. DNA analysis of endometrial tumor showed that FOXP3 gene promoter was methylated in 71% of cases. The number of cases with positive methylation status was increasing with lower differentiation grade, that was associated with the low number of FOXP3+-tumor cells. Statistically significant correlation ($p < 0.05$) (Spearman rank correlation) was observed between the deep invasion of tumor in myometrium and the number of FOXP3+-tumor cells ($R = -0.63$), number of FOXP3+- and CD4+-lymphocytes ($R = 0.68$ and$R = -0.55$, respectively) as well as the level of tumor proliferative activity ($R = 0.74$).

Conclusion: Quantitative changes of some components of the tumor microenvironment such as CD4+-, CD8+-, FOXP3+-lymphocytes and content of FOXP3+-tumor cells correlate with the biological characteristics of EC and apparently have a significant role in the progression of this cancer.

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P131

Overall survival of head and neck cancer patients of Tomsk region

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Background: The aim of the study was to identify the factors that determine outcome and overall survival of HNC patients of Tomsk region.

Materials and methods: Clinical data, morphological characteristics of tumors and outcomes were obtained for 91 patients. Data about lifestyle, food preferences, smoking history were obtained from the questionnaire ($n = 35$). All clinical samples were tested by AmpliSens HPV diagnostic kits (Russia) to determine prevalence of 12 high risk HPV types. Statistical analysis was performed using Kaplan–Meier method, Cox regression, Gehan test, Fisher test, Mann–Whitney and Kruskal–Wallis tests.

Results: Gender, age, smoking status, alcohol consumption, distance from harmful factors, professional hazards and duration of its exposure as well as tumor criteria such as T, N, G, the presence of keratinization, invasion into the underlying tissues, HPV-infection, chemotherapy and/or radiation therapy and response to the treatment were assessed for HNC patients. It was shown that the two-year survival rate was about 70%, and the five-year survival rate was about 32%. There was strong correlation between decreased overall survival and increased alcohol consumption ($p = 0.03$) as well as regional lymph nodes status ($p = 0.01$). Patients with early tumor stages and N0 lymph node status as well as patients receiving chemotherapy and/or radiotherapy showed trend towards to better survival ($p = 0.09$, $p = 0.1$, $p = 0.09$, respectively). Overall survival of patients with lymph node metastasis was higher in case of early tumor stages ($p = 0.08$) and in patients who had no alcohol consumption history ($p = 0.06$). Cox regression analysis was used to obtain the model describing overall survival of patients. The model with the highest level of significance includes 3 factors-nodal metastases, the presence of keratinization and radiotherapy. It was shown that the risk of death was 4.2 and 2.6-fold higher in case of lymph node metastases and keratinized cancer, and 2.7-fold lower in case of radiotherapy. It was also shown that metastasis occurred more frequently in cases with invasion into the underlying tissue of a primary tumor ($p = 0.04$) and in cases with a low tissue grade ($p = 0.02$). Association of alcohol consumption with questionnaire data was studied. It was shown that men's preferably smokers consume alcohol more often than other patients ($p = 0.006$, $p = 0.02$, respectively). Our data showed that HPV prevalence was higher in smokers ($p = 0.04$), and in patients with early tumor stages ($p = 0.07$). Furthermore, response to radiotherapy was better in HPV-positive patients compared to HPV-negative cases ($p = 0.09$). Better response to radiotherapy showed the group of patients who received dose higher than 45 Gy ($p = 0.03$) and who had no lymph node metastases($p = 0.05$).